

# Utilization of distillery wastewater for hydrogen production in one-stage and two-stage processes involving photofermentation

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## ARTICLE INFO

### Keywords:

Distillery wastewater  
Hydrogen production  
Purple bacteria  
Dark fermentation  
Photofermentation

## ABSTRACT

In this study, distillery wastewater was treated by dark fermentation or photofermentation alone, and by sequential dark and photofermentation processes using anaerobic saccharolytic consortium and purple nonsulfur bacteria. Combination of dark and photofermentation resulted in the maximal H<sub>2</sub> yield of 17.6 L/L of distillery waste with chemical oxygen demand 40 g/L. It is equivalent to 205 kJ/L distillery wastewater and corresponds to recovery of approximately 4–8% of energy consumed during ethanol production. Optimal performance of photofermentation was observed at 20% concentration of pre-fermented distillery waste. In photofermentation, the range of the suitable distillery waste concentrations was extended and the H<sub>2</sub> yield was improved by choosing the tolerant strain of purple bacteria *Rhodobacter sphaeroides* B-3059. After two stages, organic acids and sugars were completely consumed that means wastewater treatment concomitant to H<sub>2</sub> production.

## 1. Introduction

Biotechnological processes offer an economic and versatile way to transform resources from wastewater into valuable products, which is a prerequisite for the development of a cradle-to-cradle bio-based economy [1]. Several technologies are considered to convert organic matter to bioenergy, such as biohydrogen, biodiesel, bioethanol, and microbial fuel cell technology. Hydrogen as a clean energy carrier is receiving much attention nowadays due to the depletion of natural resources, particularly fossil fuels. Although the current biohydrogen yields are low, recent achievements in biotechnology and genetic engineering allow us to expect a rapid increase in amount of generated H<sub>2</sub> [2]. Biological H<sub>2</sub> production can be both autotrophic (biophotolysis) and heterotrophic (dark and photofermentation). Dark fermentation of wastes with concomitant H<sub>2</sub> production has been reported for a long time and is quite topical (see for example, [3,4]). Photofermentation is carried out by purple nonsulfur bacteria (PNSB),<sup>1</sup> which use light as an energy source and various organic compounds as electron and carbon sources. There are numerous examples of their application in treatment of various wastewater (domestic, sago starch processing, sardine processing, production of tofu, soybean, olive mill etc) reviewed by Keskin et al. [5]. The growth of PNSB resulted in the improvement of wastewater quality and even in H<sub>2</sub> production thus providing the cheap

energy source [5,6].

One of the most abundant organic containing wastes is the distillery wastewater (DWW) generated from alcohol distilleries. Ethanol distillery is a very energy consuming process. Besides, an estimated 8–15 L of DWW is generated per 1 L of alcohol produced [7]. The increasing generation of DWW brings about the environmental pollution problems. Thus, the treatment of DWW with a possibility of energy recovery is a vital task. DWW is characterized by high biochemical oxygen demand (BOD) at 50–60 g/L and chemical oxygen demand (COD) at 110–190 g/L. It also contains inorganic N, P, K, Ca, and S compounds [8]. A number of physicochemical and biological approaches (or their combinations) to address this issue was reviewed by Mohana et al. [8]. The possibility of DWW processing with simultaneous H<sub>2</sub> production appears to be particularly promising.

The dark anaerobic DWW fermentation was studied in several works. Mixed microbial cultures at mesophilic temperature of 37.5 °C were used in fermentation of DWW in sequential batch cycles [9]. Vatsala et al. [10] demonstrated dark fermentation of DWW by mixed culture of *Citrobacter freundii*, *Enterobacter aerogenes*, *Rhodopseudomonas palustris* in large-scale (up to 100 m<sup>3</sup>) process. The reported H<sub>2</sub> yield was 2.76 mol/mol glucose [10].

DWW was also used in light-dependent growth of PNSB or even H<sub>2</sub> production [11–14]. To meet the requirements for nitrogenase

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<sup>1</sup> PNSB, purple nonsulfur bacteria; DWW, distillery wastewater; BOD, biochemical oxygen demand; COD, chemical oxygen demand; VFAs, volatile fatty acids; BChl, bacteriochlorophyll; PhBR, photobioreactor.

synthesis, the C/N ratio in DWW must be appropriate. However, the complex nature of wastes does not allow to estimate this parameter because it is difficult to reveal all the particular C- and N-compounds utilizable by purple bacteria. The necessity to dilute DWW before processing depends on initial COD. Most often, 5–50% DWW concentration was used in batch process.

Apart from monocultures of PNSB, there is a study with hybrid one-stage photofermentation where inoculum contained both anaerobic and photosynthetic consortia [12]. This process was performed as a cyclic one with a regular substrate addition. The cumulative hydrogen production was ~ 1.5 L/L of reactor, the removal of COD and volatile fatty acids (VFAs) was 56 and 63%, respectively. Unfortunately, there was a need to replenish PNSB at regular intervals.

Photofermentation as a second step, after dark fermentation, in sequential process of different wastes treatment was explored quite extensively (see for example, [15,16]). In this case the by-products of the first stage become substrates for the second stage and in total, it is possible to get the theoretical value 12 mol H<sub>2</sub> per mole of glucose with simultaneous waste purification [17,18]. The key point is that each stage can be performed at a highest rate under specific optimal conditions. The application of this method for DWW treatment has not been reported yet.

A goal of the current study was to estimate the microbial treatment of distillery wastewater by dark fermentation, photofermentation, and integrated two-stage process in terms of energy production (as H<sub>2</sub>) and concomitant waste purification.

## 2. Materials and methods

### 2.1. Bacteria and media

The dark anaerobic saccharolytic consortium was obtained from silage pit liquid and cultivated at 37 °C as described before [19,20]. The purple non-sulfur bacteria *Rhodobacter capsulatus* B10 and *R. sphaeroides* B-3059 (previously described as strain N7 [21]) were grown on Ormerod medium with ammonium sulfate and lactate at 30 °C, light intensity 30 W/m<sup>2</sup>. For the short-term experiments, bacteria were grown on lactate-containing Ormerod medium with ammonium limitation (2 mM NH<sub>4</sub><sup>+</sup>).

### 2.2. DWW treatment

The samples of DWW were supplied by distillery plants in Kashira and Vladikavkaz (Russia) (Cristall OJSC). DWW pretreatment included neutralization to pH 6.7 with KOH, centrifugation at 5000 rpm and sterilization. Then, DWW composition was analyzed and samples were tested as substrates for photofermentation or dark fermentation.

### 2.3. Dark fermentation of DWW

Dark anaerobic fermentation of DWW (0.25 L) was carried out under batch operation in 0.50-L capped glass vials under N<sub>2</sub> gas phase at 37 °C using above mentioned consortium (inoculum volume 0.5%). The pH value was maintained above 5.0 by daily titrations with 50% KOH. Gas production was measured manometrically. When gas production stopped, the culture medium was treated similarly to initial DWW. The resulting effluent was further utilized as pre-fermented DWW (DWW<sub>f</sub>).

### 2.4. Photofermentation of DWW or DWW<sub>f</sub>

Photofermentation of the initial DWW or pre-fermented DWW<sub>f</sub> was also referred to as long-term H<sub>2</sub> photoproduction (5–7 days). It was carried out in vials of total volume 30 or 500 mL (10 or 250 mL of culture, respectively) under the Ar gas phase with mixing. The 300-mL vials were also referred to as photobioreactors (PhBRs). Cultivation

temperature was 30 °C, incident light intensity 30 W/m<sup>2</sup>. Two species of PNSB, *R. capsulatus* B10 or *R. sphaeroides* B-3059, were used as an inoculum (5%). The gas production in PhBRs was recorded by gas-water displacement. The gas production in 30-mL vials was measured manometrically. The H<sub>2</sub> percentage in gas was determined by GC [22].

### 2.5. Short-term H<sub>2</sub> photoproduction

Short-term (≤2h) H<sub>2</sub> production from DWW was performed in 15-mL vials (2 mL of culture) under Ar gas phase at 30 °C, incident light intensity 30 W/m<sup>2</sup>. H<sub>2</sub> production was followed by GC as described above.

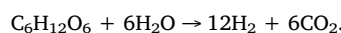
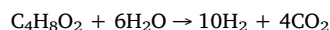
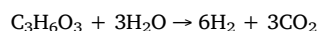
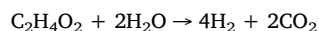
### 2.6. Other analytical methods

The concentrations of acetate, propionate, butyrate and isobutyrate were determined by gas chromatography as described earlier [22]. Lactate concentrations were determined by an enzymatic method [23]. Bacteriochlorophyll (BChl) concentration was measured spectrophotometrically at 772 nm after extraction in 7:2 (v/v) acetone:methanol mixture [24]. The total content of soluble saccharides was measured using anthrone reagent after hydrolysis by sulfuric acid and presented as glucose equivalents [25]. The ammonium content was analyzed by microdiffusion method [26]. Protein concentration was estimated according to Lowry method. COD was measured by potassium dichromate method.

All results represent the mean value of three experiments ± 95% confidence interval.

### 2.7. Calculation of H<sub>2</sub> production efficiency

The efficiency of hydrogen production (VFAs and glucose transformation into H<sub>2</sub>) was calculated as the ratio of the actual H<sub>2</sub> production to the theoretical hydrogen production. The latter was calculated assuming that all utilized VFAs (acetate, lactate, butyrate) and glucose can be converted to H<sub>2</sub> according to known stoichiometry:



## 3. Results and discussion

### 3.1. Some properties of two DWW samples

Two samples of DWW were supplied from different distillery plants (Section 2.2). The composition of these samples was similar: soluble saccharides (glucose residues) ~5–6 g/L, protein ~3–5 g/L, total VFAs ~ 19–24 mM (mainly lactate) (Table 1). There were only traces of ammonium but perceptible amounts of protein. Excess of ammonium is often a problem for photofermentation of different wastes if the process aims to H<sub>2</sub> production [5], but it was not a case when using DWW. However, the presence of proteins (amino acids) and some other N-compounds could influence nitrogenase expression as well. COD in DWW2 was about 40 g/L.

Given the data in Table 1, one can estimate the theoretical H<sub>2</sub> production (yield) by PNSB on the base of known stoichiometry (see Section 2.7). The theoretical H<sub>2</sub> production from VFAs is 2.4 or 3.5 L per 1 L of DWW1 or DWW2, respectively. In addition, glucose equivalents could provide 8.2 or 9.5 L per 1 L of DWW1 or DWW2, respectively. However, the precise chemical composition of anthrone-reactive substances (glucose equivalents) in DWW is unknown. It is unclear whether they are suitable as electron donors for H<sub>2</sub> production by

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